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Amniotic fluid stem cells within chimeric kidney rudiments differentiate to functional podocytes following transplantation into mature rat kidneys

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The pioneering work of Grobstein, Saxen and Sariola, led to the development of culture systems that could support the growth and differentiation of mouse kidney rudiments *in vitro*.[1] Cultured kidney rudiments can be easily manipulated, either genetically or by the application of growth factors, inhibitors, or other small biological substances, and are thus useful model systems for investigating the mechanisms that regulate the development of the mammalian kidney. Kidney rudiments have also been evaluated for their potential to integrate into mature kidneys and treat renal insufficiency. Woolf *et al.* showed that rudiments transplanted into neonatal mouse kidneys generated functional nephrons with filtering capacity,[2] with subsequent studies by Rogers and Hammerman demonstrating that transplanted rudiments could improve survival in anephric rats, albeit for only a few days.[3] However, despite these promising results, further studies by Ashton and co-workers showed that while the kidney rudiments continued to develop for a few weeks following transplantation, by three months, their level of maturity only reached that of early neonatal kidneys.[4] This meant that the glomerular filtration rate (GFR) of the transplanted rudiments was equivalent to only 2% of the GFR of an adult rat kidney, which explains why the rudiments were unable to sustain life in anephric rodents beyond a few days.

Although these studies suggest that kidney rudiments are unlikely to have much therapeutic value when transplanted into diseased kidneys, they can nevertheless be very effective tools for assessing the nephrogenic potential of various types of stem and progenitor cells. For instance, following on from the work of Atala and co-workers,[5] who found that disaggregated metanephroi isolated from bovine embryos could self-organise to form functioning nephrons, Unbekandt and Davies developed an assay that involved disaggregating mouse kidney rudiments to single cells, combining them with exogenous stem/progenitor cells, and then allowing the cells to re-aggregate to form chimeric kidney rudiments.[6] These chimeric rudiments could be cultured *in vitro*, presenting excellent test systems for assessing whether the exogenous stem cells are capable of

generating specialized renal cells.[6] Using this assay, it has been shown that certain types of stem cells, most notably, pluripotent stem cells[7] and amniotic fluid stem cells (AFSCs),[8] are capable of integrating into developing renal structures and generating specialized renal cells, whereas other cell types, such as mesenchymal stem/stromal cells (MSCs) are unable to do so,[9] unless they have been engineered to over-express glial cell line-derived neurotrophic factor (GDNF).[10]

The chimeric kidney rudiment assay therefore allows researchers to identify stem cell types with nephrogenic potential that could potentially be used for applications in regenerative medicine, drug discovery and disease modelling. However, although the chimeric rudiment assay is very useful for investigating whether different stem cell types can participate in nephron development, and can even be used with great effect to test the functionality of stem cell-derived proximal tubule cells,[7] a major drawback with the *in vitro* kidney rudiment system is that endothelial cells do not invest the developing glomeruli, and consequently, there is an absence of capillary loops, resulting in the failure of podocyte maturation. Furthermore, the lack of vasculature means that the podocytes are unable to perform selective filtration as they would *in vivo*, and hence, the functionality of stem cell-derived podocytes cannot be tested. However, a study by Xinaris *et al.*[11] has shown that if re-aggregated rudiments, also referred to as kidney 'organoids', are transplanted into adult rat kidneys, they can generate vascularised glomeruli with capillary loops and slit diaphragms that appear to have some filtration capacity.

In this issue of JASN, Xinaris *et al.*[12] have extended these studies to (i) explore whether re-aggregated mouse rudiments can perform ultrafiltration; (ii) assess the nephrogenic potential of hAFSCs within chimeric rudiments comprised of re-aggregated mouse metanephroi; (iii) determine whether the hAFSCs within the chimeras can generate mature, functional podocytes following transplantation into mature rat kidneys. Using electron microscopy, the authors first show that

following transplantation into the kidneys of uni-nephrectomised athymic adult rats, re-aggregated mouse kidney rudiments could generate vascularized glomeruli, some of which contained mature podocytes with well-developed foot processes and slit diaphragms. The functionality of the glomeruli was then investigated using fluorescently labelled dextrans of low (10 kDa, 70 kDa) and high (155 kDa) molecular weight. Some of the nephrons that formed within the chimeric rudiments were found to be capable of performing ultrafiltration, as evidenced by their ability to filter the low molecular weight dextrans, but exclude the high molecular weight dextran. The authors then evaluated the nephrogenic potential of the hAFSCs. In contrast to a previous study,[8] the authors showed that hAFSCs within chimeric rudiments cultured *in vitro* had a limited capacity to integrate into developing renal structures, but if genetically modified with an adenovirus vector encoding GDNF, they could become incorporated into the condensed metanephric mesenchyme. When chimeric rudiments containing GDNF-expressing hAFSCs were cultured for 1 day *in vitro* and then transplanted into mature rat kidneys, immunofluorescence analysis showed that the hAFSCs could readily form podocytes. The ability of the hAFSCs to form mature podocytes was confirmed using immuno-electron microscopy, which showed that the hAFSC-derived podocytes had well-developed foot processes and slit diaphragms. Finally, to demonstrate functionality, dual immunogold staining for a human-specific antigen and bovine serum albumin was performed to show that hAFSC-derived podocytes were able to endocytose albumin. An interesting finding of the Xinaris *et al.* study is that the hAFSCs had a marked propensity to form podocytes, and rarely formed proximal tubule cells. The reasons for this are not entirely clear, but the authors obtained the same result using two different hAFSC lines, indicating that the tendency of the hAFSCs to form podocytes was not a peculiarity of any one particular hAFSC line.

An important aspect of the Xinaris *et al.* study[12] is that it shows how transplanted chimeric

rudiments could potentially be used to model diseases affecting human podocytes. For instance, the use of hAFSCs with mutations in genes that affect podocyte differentiation, growth, survival and/or function, would allow detailed studies of the processes of disease initiation and progression, and the evaluation of possible therapies. Furthermore, by using patient-derived human induced pluripotent stem cells (hiPSCs) instead of hAFSCs, it would be possible to investigate a wider range of human kidney diseases, due to the fact that unlike hAFSCs, iPSCs cultured under the appropriate conditions can generate the full suite of cell types that comprise the nephron.[13]

Could chimeric kidney organoids be used to treat renal insufficiency? At present, this seems unlikely, because in addition to the aforementioned issues with low GFR that have been reported following the transplantation of intact rudiments,[4] in the re-aggregated chimeric rudiments, there is the additional problem of the ureteric bud tree not being continuous, making it unable to transfer urine from the cortico-medullary region to the renal pelvis.

In conclusion, Xinaris *et al.*[12] report that if hAFSCs are engineered to express GDNF and are then combined with disaggregated mouse kidney rudiments to form chimeric kidney organoids, the hAFSCs can differentiate to form functional podocytes following transplantation into adult rat kidneys. The authors' technology could potentially be used as a model for understanding diseases affecting the podocytes, and as a system for testing novel therapies to treat podocyte disease.

Disclosures

None.

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